Heterogeneity of Cytochrome P-450s Induced by Polychlorinated Biphenyls*

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Alvito P. Alvarés† and Attallah Kappas

From the Rockefeller University, New York, New York, 10021

The polychlorinated biphenyl mixture, Aroclor 1254, is a powerful inducer of both cytochromes P-450 and P-448 exhibiting the catalytic properties of both cytochromes in the liver. Addition of Aroclor 1254 to hepatic microsomes produces a type I difference spectrum. When administered to rats, the hemeprotein induced by Aroclor 1254 displayed spectral properties which are a combination of the spectral properties of the two cytochromes. The absorption maximum of the CO complex of the hemeprotein, the ratio of the 455 to 430 nm peaks of the ethyl isocyanide complex, and the pH intercepts obtained by the dependence of these peaks on the pH are intermediate between those obtained with cytochromes P-450 and P-448. Partially purified hemeproteins from Aroclor 1254-treated rats exhibited a CO spectrum with an absorption maximum at 418 nm. The catalytic properties of the induced hemeproteins are a combination of the catalytic properties of cytochrome P-450 induced by phenobarbital and of cytochrome P-448 induced by 3-methylcholanthrene in rats. The biphenyl mixture markedly enhanced the cytochrome P-450-associated enzymes, including the ethylmorphine and benzphetamine N-demethylases and the hexobarbital oxidative pathway. Similarly, the cytochrome P-448 enzyme activities, including the hydroxylation of benz(o)pyrene and of oxazolactone were greatly enhanced. Aroclor 1254 also increased cytochrome b, and NADPH-cytochrome c reductase activity of liver microsomes. Enhancement of benz(o)pyrene hydroxylase activity occurred in nonhepatic tissues, including lung, kidney, adrenals, intestine, and testis. Acute exposure of rats to Aroclor 1254 resulted in a small but significant induction of &-aminolevulinic acid synthetase and inhibition of &-aminolevulinic acid dehydratase but no significant accumulation of porphyrins in the liver. These results clearly demonstrate that the polychlorinated biphenyl mixture, Aroclor 1254, possesses the inducing properties of the two main classes of microsomal enzyme inducers, namely phenobarbital and 3-methylcholanthrene.

Polychlorinated biphenyls are used in electrical capacitors and transformers, heat transfer systems, hydraulic lubricants, and other miscellaneous applications. They have gained access into the environment from leaks, spills, vaporization during manufacturing processes, and from disposal waste. They tend to accumulate in the food chain and once ingested are stored in the body's fatty tissues. PCBs have been detected in the tissues of numerous animals, fish, and birds (1-3), as well as in human adipose tissue (4) and in human breast milk (5). Previous investigations have shown that PCBs are inducers of steroid hydroxylases (6) and induce the metabolism by hepatic monoxygenases of a variety of substrates, including drugs (7-9) and polycyclic hydrocarbon carcinogens (7, 8).

Inducers of hepatic monoxygenases have been categorized into two main groups (10). One group of inducers of which phenobarbital is a prototype, enhances the metabolism of a large variety of substrates by these liver enzymes; a second group stimulates the metabolism of only a few substrates. Polycyclic hydrocarbons, such as benz(o)pyrene and 3-methylcholanthrene comprise the second group of compounds. In addition, the effects of these two groups of inducers on cytochrome P-450, the terminal oxidase in the liver that metabolizes drugs, carcinogens, and other foreign compounds are considerably different. 3-Methylcholanthrene induces the synthesis of cytochrome P-448, a hemeprotein that differs in spectral and catalytic properties from cytochrome P-450 (10-12). Previous studies from this laboratory (7) have shown the PCBs are potent inducers of ethylmorphine N-demethylase activity, an enzyme preferentially induced by phenobarbital, and of benz(o)pyrene hydroxylase activity, an enzyme preferentially induced by the polycyclic hydrocarbon class of inducers, such as 3-methylcholanthrene. However, the hemeprotein induced by the PCBs showed an absorption maximum at 448 to 449 nm. These data indicated that PCBs may be unique in that they induced cytochrome P-448 which was catalytically different from the 3-methylcholanthrene-induced P-448 or that the hemeprotein(s) induced by PCBs may be a mixture of cytochromes P-450 and P-448 exhibiting catalytic properties of both cytochromes.

In the present study, we have further investigated the prop-

1 The abbreviations used are: PCBs, polychlorinated biphenyls; &-ALA, &-aminolevulinic acid.
2 The constitutive and inducible hemeproteins, cytochromes P-450 and P-448 (also called P-450), may represent more than one type or species of CO-binding hemeproteins. These species are detectable by gel electrophoresis (11-13) of the purified hemeproteins.
Induction of Microsomal Heme Proteins by Chlorinated Biphenyls

Spectral Interactions of Aroclor 1254 with Liver Microsomes—Drugs and other foreign compounds which are substrates for hepatic cytochrome P-450 bind with the heme protein to produce difference spectra of two general types, type I and type II (29). Type I compounds give a difference spectrum with a maximum in the range of 385 to 390 nm and a minimum in the range of 418 to 427 nm; for type II compounds a minimum at 425 nm and a maximum at 390 to 405 nm, respectively. As shown in Fig. 1, addition of Aroclor 1254 to liver microsomes, gave a typical type I difference spectrum with a maximum at 388 nm and a minimum at 424 nm. These data indicate that component(s) of the PCBs bind to cytochrome P-450 and may serve as substrates for the microsomal hemoeprotein.

Changes in Spectral Properties of Cytochrome P-450 Induced by Aroclor 1254—Administration of Aroclor 1254 to rats, besides causing a marked increase in cytochrome P-450 content in liver cells, causes several changes in the spectral properties of the hemoeprotein detected when various ligands are used (Table I). Also in Table I is a comparison of the spectral properties of the hemoeprotein induced by phenobarbital, 3-methylcholanthrene, and Aroclor 1254. These data show that the hemoeprotein induced by Aroclor 1254 shares the spectral properties of the hemoeproteins induced by phenobarbital as well as 3-methylcholanthrene. Like the 3-methylcholanthrene-induced P-448, Aroclor 1254-induced hemoeprotein shows a shift in the absorption maximum to 448 to 449 nm in the CO difference spectrum, a shift in the absorption maximum of the 455 nm peak of the ethyl isocyanide-induced difference spectrum and causes a change in the ratio of the 455 to 430 nm peaks of the ethyl isocyanide difference spectrum. The relative heights of the 455 nm and 430 nm spectral peaks of the ethyl isocyanide difference spectra have been previously shown to be pH-dependent (30). Previous studies have shown that if the peaks heights of the 455 and 430 nm peaks are plotted independently against the pH of the microsomal suspensions, the plots intercept is significantly lower when microsomes from 3-methylcholanthrene-treated rats are used when compared to the intercept obtained with untreated or phenobarbi-
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As shown in Table I, the pH intercept obtained with microsomes from Aroclor 1254-treated rats, like the ratio of the 455 to 430 nm peaks, is intermediate between those obtained with phenobarbital- and 3-methylcholanthrene-induced microsomes from untreated, phenobarbital-, or Aroclor 1254-treated rats. Hexobarbital added to oxidized microsomes from untreated, phenobarbital-, or Aroclor 1254-treated rats gave a typical type I difference spectrum whereas 3-methylcholanthrene microsomes exhibited a modified type II difference spectrum, as has been previously reported (31), with a \( \lambda_{\text{max}} \) at 417 nm and a \( \lambda_{\text{min}} \) at 387 nm. Aniline gave a typical type II difference spectrum with microsomes from untreated as well as with variously treated microsomes. Thus, in sum, the spectral properties of the hemeprotein induced by Aroclor 1254 appear to be a combination of the properties exhibited by cytochrome P-450 induced by phenobarbital and cytochrome P-448 induced by 3-methylcholanthrene.

### Table I

Comparison of changes in spectral properties of cytochrome P-450 induced by phenobarbital, Aroclor 1254, and 3-methylcholanthrene

<table>
<thead>
<tr>
<th>Assay</th>
<th>Controls</th>
<th>Phenobarbital</th>
<th>Aroclor 1254</th>
<th>3-Methylcholanthrene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CO difference spectrum: absorption maximum, nm</td>
<td>450</td>
<td>450</td>
<td>448-449</td>
<td>448</td>
</tr>
<tr>
<td>2. Ethyl isocyanide difference spectrum: absorption maximum of 455 nm peak</td>
<td>455</td>
<td>455</td>
<td>453-454</td>
<td>453</td>
</tr>
<tr>
<td>3. Ethyl isocyanide difference spectrum: ( \lambda_{\text{max}} ) ( \lambda_{\text{min}} )</td>
<td>0.6</td>
<td>0.8</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td>4. Ethyl isocyanide difference spectrum: pH intercept</td>
<td>7.7</td>
<td>7.5</td>
<td>7.1</td>
<td>6.9</td>
</tr>
<tr>
<td>5. Hexobarbital-induced difference spectrum</td>
<td>Type I(a)</td>
<td>Type I(a)</td>
<td>Type I(b)</td>
<td>Modified(c) type II</td>
</tr>
<tr>
<td>6. Aniline-induced difference spectrum: Absorption maximum, nm</td>
<td>Type II(a)</td>
<td>Type II(a)</td>
<td>Type II(a)</td>
<td>Type II(a)</td>
</tr>
</tbody>
</table>

\( a \) \( \lambda_{\text{max}} \) 385 to 390 nm; \( \lambda_{\text{min}} \) 418 to 422 nm.

\( b \) \( \lambda_{\text{max}} \) 417 nm; \( \lambda_{\text{min}} \) 387 nm.

\( c \) \( \lambda_{\text{max}} \) 425 to 435 nm; \( \lambda_{\text{min}} \) 390 to 405 nm.

### Table II

Comparison of changes in liver microsomal enzymes induced by phenobarbital, Aroclor 1254, and 3-methylcholanthrene

<table>
<thead>
<tr>
<th>Assay</th>
<th>Controls</th>
<th>Phenobarbital</th>
<th>Aroclor 1254</th>
<th>3-Methylcholanthrene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microsomal protein, mg/g liver, wet weight</td>
<td>16.4</td>
<td>16.5</td>
<td>16.4</td>
<td>16.5</td>
</tr>
<tr>
<td>2. Cytochrome ( b_2 ) content, nmol/mg protein/h</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>3. Cytochrome P-450 content, nmol/mg protein</td>
<td>0.73</td>
<td>0.73</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>4. NADPH-cytochrome c reductase, nmol cytochrome c reduced/mg protein/min</td>
<td>118.40</td>
<td>118.40</td>
<td>118.40</td>
<td>118.40</td>
</tr>
<tr>
<td>5. Benzphetamine N-demethylase, ( \mu )mol HCHO/mg protein/h</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>6. Ethylmorphine N-demethylase, ( \mu )mol HCHO/mg protein/h</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>7. Benzo(a)pyrene hydroxylase, nmol ( \mu )mol protein/h</td>
<td>4.21</td>
<td>4.21</td>
<td>4.21</td>
<td>4.21</td>
</tr>
<tr>
<td>8. Aniline hydroxylase, nmol p-aminophenol/mg protein/h</td>
<td>36.42</td>
<td>36.42</td>
<td>36.42</td>
<td>36.42</td>
</tr>
<tr>
<td>9. Hexobarbital-induced sleeping time, min</td>
<td>109</td>
<td>113</td>
<td>113</td>
<td>113</td>
</tr>
<tr>
<td>10. Zoxazolamine-induced paralysis time, min</td>
<td>582</td>
<td>582</td>
<td>582</td>
<td>582</td>
</tr>
</tbody>
</table>

\( a \) Value significantly different from respective control value (\( p < 0.05 \)).
mixture should not have an additive effect when the inducer is administered in combination with phenobarbital or 3-methylcholanthrene. In Fig. 2A, it can be seen that the increase in ethylmorphine N-demethylase activity, a P-450-associated enzyme, observed when maximal stimulatory doses of Aroclor 1254 or phenobarbital are administered, was similar to that observed when the PCBs mixture is administered concurrently with phenobarbital. Similarly, as shown in Fig. 2B, the PCBs-induced increase in benzo(a)pyrene hydroxylase activity, a P-448-associated enzyme, was not additive when Aroclor 1254 was administered concurrently with 3-methylcholanthrene; however, when Aroclor 1254 and phenobarbital were administered concurrently, the induction of the hydroxylase activity was additive. Similarly, in Fig. 2C, it can be seen that NADPH-cytochrome c reductase, which is inducible by Aroclor 1254 and phenobarbital but not by 3-methylcholanthrene, did not result in any additive effects when Aroclor 1254 is administered in combination with phenobarbital. As can be seen in Fig. 2D, the ratio of the 455 to 430 nm peaks observed with Aroclor 1254 is intermediate between the ratio observed with phenobarbital and 3-methylcholanthrene. Administration of Aroclor 1254 with phenobarbital resulted in an increase in ratio similar to that observed with Aroclor 1254 alone; whereas the administration of Aroclor 1254 with 3-methylcholanthrene resulted in the ratio being equal to that obtained when 3-methylcholanthrene was administered alone. These studies provide further evidence that Aroclor 1254 causes the synthesis of P-450 and P-448 independently.

Induction of Aryl Hydrocarbon Hydroxylase in Nonhepatic Tissues by Aroclor 1254 – A major characteristic of the 3-methylcholanthrene class of inducers is its ability to induce aryl hydrocarbon (benzo(a)pyrene) hydroxylase activity in a variety of nonhepatic tissues (34). Phenobarbital is a poor inducer of the hydroxylase activity in the liver and does not cause any significant induction in nonhepatic tissues (34). If Aroclor 1254 possessed the inducing characteristics of cytochrome P-448-associated benzo(a)pyrene hydroxylase, it should be a potent inducer of the hydroxylase activity in nonhepatic tissues. This was indeed the case as shown in Fig. 3. The 10- to 15-fold induction of benzo(a)pyrene hydroxylase activity occurred in homogenates of liver, kidney, and intestine of rats pretreated with Aroclor 1254. Lung, testis, and adrenals showed about 5-fold increases in the hydroxylase activity. Previous studies have shown that PCBs also have a marked stimulatory effect on skin (35) and placental (36) benzo(a)pyrene hydroxylase activity.

Spectral Properties of Partially Purified Cytochrome P-450 from Aroclor 1254-treated Rats – In order to further examine the hemeprotein(s) induced by the PCBs, a partially purified preparation of the hemeprotein induced in rats pretreated with Aroclor 1254 was prepared by the method of Lu and Levin (19) and carried through the calcium phosphate gel step of their method. The absolute spectra of the induced hemeprotein are shown in Fig. 4. The oxidized spectrum had a Soret band at 416 nm and absorption bands at about 537 and 568 nm. When the oxidized form was reduced with sodium dithionite, the Soret peak shifted downward to 414 nm and a broad peak was observed at around 650 nm. Those spectra are very similar to those which Levin et al. (37) have reported with their partially purified P-450 and P-448 preparations derived from phenobarbital- and 3-methylcholanthrene-treated rats, respectively. The CO spectrum of the reduced hemeprotein showed a small peak at 422 nm and an absorption maximum of 448 nm. The peak at 422 nm may be due to the presence of contaminating amounts of cytochrome b5 or due to the presence of cytochrome P-420, the inactive form of P-450. The occurrence of the absorption maximum at 448 nm in the CO spectrum of the reduced hemeprotein provides further evidence that the hemeproteins induced by Aroclor 1254 are a mixture of cytochromes P-450 and P-448, since studies by Ryan et al. (36) show that the spectral maxima for P-450 purified from phenobarbital-treated rats.
Induction of Microsomal Hemeproteins by Chlorinated Biphenyls

DISCUSSION

The present studies conclusively demonstrate that the PCBs mixture Aroclor 1254 is a powerful inducer of both cytochromes P-450 and P-448 exhibiting the catalytic properties of both cytochromes in the liver. When administered to rats, PCBs evoke the inducing properties of both the phenobarbital and the 3-methylcholanthrene classes of inducers. Previous studies of this laboratory have shown that when the apoproteins of these cytochromes are subjected to slab gel electrophoresis, three major bands with molecular weights of 47,000, 49,000, and 52,000 are observed. This pattern resembled an additive combination of the 47,000 and 49,000 bands observed with the hemeproteins purified from phenobarbital-treated rats and the 52,000 molecular weight band from 3-methylcholanthrene-treated rats (11). In the present studies, the spectral properties displayed by the induced hemeproteins in microsomes are as high as in partially purified preparations of the hemeproteins are shown to be a combination of the spectral properties of the cytochromes P-450 and P-448. The catalytic properties of the hemeproteins induced by Aroclor 1254 are also similar to those observed with the hemeproteins from phenobarbital and 3-methylcholanthrene-induced rats. When Aroclor 1254 was administered in combination with phenobarbital at maximally stimulatory doses, there was no additive effect on the inducibility of ethylmorphine N-demethylase and on NADPH-cytochrome c reductase observed when each of these inducers was administered alone. Similarly, there was no additive effect when Aroclor 1254 was administered concurrently with 3-methylcholanthrene on the induction of benz(a)pyrene on the induction of benzo(a)pyrene hydroxylase activity and on the increase in the ratio of the 455 to 430 nm peaks of the ethyl isocyanide difference spectrum of cytochrome P-450 were maximally increased in these experiments. In addition, like 3-methylcholanthrene, Aroclor 1254 was a potent inducer of aryl hydrocarbon hydroxylase in a number of nonhepatic tissues. Finally, the hemeprotein(s) which were partially purified from Aroclor 1254-purified rats to Aroclor 1254 does not cause porphyria or the induction of hepatic heme and cytochrome P-450 content.

Effects of Aroclor 1254 on Heme Biosynthetic Enzyme Activities—Since the long term administration of PCBs to rats has been shown to cause hepatic porphyria (38), it was of interest to determine if PCBs administered acutely results in alteration of enzymic activities in the heme metabolism pathway. As shown in Table III, acute administration of Aroclor 1254 resulted in a rather small but significant increase in δ-ALA synthetase, the rate-limiting enzyme of heme biosynthesis pathway. On the other hand, δ-ALA dehydratase was inhibited, but these changes in enzymic activities did not result in any accumulation of liver porphyrins. In sum, Aroclor 1254 administered acutely possesses the properties of phenobarbital and 3-methylcholanthrene, by markedly increasing microsomal heme and cytochrome P-450 levels without significant stimulation of δ-ALA synthetase.

**DISCUSSION**

**Table III**

Effects of Aroclor 1254 on heme biosynthetic enzyme activities and on hepatic heme and cytochrome P-450 content.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Controls</th>
<th>Aroclor 1254</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ-ALA synthetase, nmol δ-ALA formed/g liver/h</td>
<td>96.51 ± 3.60</td>
<td>38.19 ± 9.77</td>
</tr>
<tr>
<td>δ-ALA dehydratase, nmol PBG* formed/mg protein/h</td>
<td>3.8 ± 0.4</td>
<td>2.31 ± 0.2</td>
</tr>
<tr>
<td>Total porphyrins, pmol/mg protein</td>
<td>5.49 ± 0.50</td>
<td>4.83 ± 0.38</td>
</tr>
<tr>
<td>Ferrochelatase, nmol mezo-heme formed/mg protein/h</td>
<td>9.12 ± 1.14</td>
<td>7.26 ± 0.54</td>
</tr>
<tr>
<td>Microsomal heme, nmol/mg protein</td>
<td>1.017 ± 0.168</td>
<td>3.538 ± 0.089</td>
</tr>
<tr>
<td>Cytochrome P-450, nmol/mg protein</td>
<td>0.836 ± 0.125</td>
<td>3.037 ± 0.245</td>
</tr>
</tbody>
</table>

* Value significantly different from the control value (p < 0.05).
*b P&g; porphobilinogen.

The present studies demonstrate that acute exposure of rats to Aroclor 1254 does not cause porphyria or the induction of δ-ALA synthetase. However, there was a significant decrease in δ-ALA dehydratase. Although the heme biosynthetic enzymes were minimally affected, microsomal heme and cytochrome P-450 were maximally increased in these experiments. These findings suggest that the synthesis of cytochromes P-450 and P-448 is regulated by factors other than a reciprocal rela-
tionship between total hepatic heme and δ-ALA synthetase activity.

Recent studies by Vos et al. (41) and by Bowes et al. (42) have shown the presence of chlorinated dibenzofurans as contaminants of various PCBs mixtures. Although significant levels of these contaminants were present in PCBs manufactured in Europe, only trace amounts of the order of 1 to 2 μg of dibenzofurans/g have been detected in the American manufactured mixture Aroclor 1254 (42). In the present studies, these amounts would be equivalent to a maximum total dosage of 0.5 μg/kg of body weight for the 6-day treatment period. The question arises whether the effects observed with Aroclor 1254 may be due to the contaminating amounts of dibenzofurans in the PCBs mixture. This does not appear to be so, since recent studies by Goldstein et al. (43) have shown that 2,3,7,8-tetrachlorodibenzofuran, the main contaminant of Aroclor 1254, unlike chlorinated biphenyls, does not induce hepatic porphyrin, has little effect on cytochrome P-450 or microsomal glucuronyltransferase activity and does not induce δ-ALA synthetase when administered to chicks at a dosage of 1 μg/kg/day for 21 days. The possibility may also be considered that the observed effects with the PCBs may be due to the presence of tetrachlorodibenzo-p-dioxin. However, this compound has never been detected in the various PCBs mixtures, including Aroclor 1254.

The ability of the PCBs to modify cytochrome P-450-mediated microsomal enzyme activities involved in drug and carcinogen metabolism may have considerable physiological implications for populations that are occupationally exposed to these long lived pollutants. Since endogenous steroid hormones also serve as substrates for the cytochrome P-450 system, it would be of considerable importance to determine the effects of PCBs which are potent inducers of cytochromes P-450 and P-448 on endogenous hormone metabolism in man since PCBs have been shown to be potent inducers of steroid hydroxylase in avian species (6).

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